Progress Toward HIV Eradication: Case Reports, Current Efforts, and the Challenges Associated with Cure

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Abstract

An estimated 35 million people worldwide are infected with HIV, yet a widely applicable cure strategy remains elusive. Recent case reports have suggested that curing HIV infection is possible, renewing excitement about research efforts. We describe those cases and discuss their relevance to the global HIV epidemic. We also review ongoing cure strategies that are transitioning from the lab to the clinic, and the assays and clinical assessments that can be used to evaluate cure interventions.

INTRODUCTION

Infection with HIV is a continuing global health concern, with ~1.5 million AIDS-related deaths worldwide in 2013. Current combination antiretroviral therapy (cART) reduces viremia to undetectable levels, preventing further loss of $CD4^+$ T cells and progression to AIDS. The use of cART allows HIV-infected individuals to have near-normal life expectancy (1). However, cART is not curative, and infected individuals must remain on therapy for life to maintain their health. We review for the nonspecialist the need for a cure, the barriers to achieving this goal, exciting recent cases in which a cure has been achieved or nearly achieved, and strategies to make HIV infection curable.

THE NEED FOR A CURE

As therapy for HIV infection has improved, the need for curative interventions, particularly those that pose risks to the patient, has been questioned. The benefits of a cure are best understood in the context of the global epidemic. According to the Centers for Disease Control and Prevention, only 36% of infected individuals worldwide are receiving cART. This is partly a financial issue, with lifetime cART estimated to cost $379,668 in 2010 (**http://www.cdc.gov/hiv/prevention/ongoing/costeffectiveness/**). The majority of HIV-infected individuals live in low-income countries and have limited access to health care. Moreover, the number of people living with HIV globally continues to increase, threatening to elevate the cost of HIV health care to unsustainable levels. Although prevention strategies have allowed the number of new HIV infections per year in the United States to remain stable, the prevalence of HIV is increasing because infected individuals live longer on cART. In addition, results from the Strategic Timing of Antiretroviral Treatment (START) trial have suggested that beginning cART as early as possible is best for long-term patient outcomes (ClinicalTrials.gov NCT00867048). These findings will likely influence patient care, broadening the indication for these drugs to all HIV-infected individuals, a prospect that will clearly be unsustainable unless a cure is found.

At the level of the individual patient, there are also reasons why a cure, even one involving some risks, would be preferable to lifetime cART. Adverse long-term effects, such as lipodystrophy, are associated with some antiretroviral drugs. Drug toxicity can decrease adherence (2). If a patient is poorly adherent or discontinues therapy without careful medical supervision, drug resistance can develop. Resistance makes it more difficult to successfully reinitiate treatment later (3). These problems contribute to the fact that only 75% infected individuals on treatment in the United States are able to maintain suppression of viral replication.

For patients able to adhere to cART, $CD4^+$ T cell counts stabilize and increase, but the immune system never fully recovers. Even patients who have been on suppressive cART for years suffer from increased immune activation and inflammation (4), which can lead to additional health problems, including cardiovascular disease. It is not clear whether these abnormalities result from legacy effects of the period of unchecked viral replication before initiation of cART or from a low-level ongoing release of virus from stable reservoirs. Cure strategies would impact the latter but not the former.

Those who are infected with HIV would also benefit from a cure for nonmedical reasons. Along with the high cost of cART, there is the social stigma of being HIV-positive, as HIV infection has historically been associated with drug use and high-risk sexual activity. Therefore, although cART has greatly improved the lives of infected individuals, there are significant problems with lifelong treatment. Clearly, a cure strategy would both benefit infected individuals and reduce the burden to the global health economy.

OBSTACLES TO A CURE

Due to a fundamental feature of HIV biology, the infection has proven difficult to cure. HIV stably integrates its genome into the DNA of the host cells it infects, predominantly $CD4^+$ T lymphocytes. A small number of these infected cells enter a state of latent infection and persist indefinitely. Cells containing a latent HIV genome can later resume active virus production. A single, integrated provirus can produce a sufficient number of virions to perpetuate infection, and when therapy is discontinued, viremia quickly rebounds (5). Thus, the persistence of latently infected cells is a major obstacle to cure. Cure strategies either aim to kill all infected cells or boost immunity to control infection. Efforts to produce a cure are accelerating, with many different strategies being studied.

Definitions

It is useful at the outset to define critical terms. Latency is a reversibly nonproductive state of infection. Latent viral infection is characterized by the presence of infected cells that do not actively produce virus particles, but retain the ability to do so in the future. A viral reservoir is a cell type that harbors replication-competent virus for long periods (6). With respect to curing HIV, the only relevant reservoirs are those that persist on a timescale of years in patients on optimal cART. This practical definition reflects the fact that cure strategies would be implemented only in patients on cART who have had suppression of active viral replication for a substantial period of time. Another related term is compartment. This refers to an anatomical site of viral replication that has limited exchange of virus with other sites.

The Latent Reservoir in Resting $CD4^+$ T Cells

A latent reservoir (LR) for HIV in resting memory $CD4^+$ T cells was demonstrated in 1995 (7, 8). The majority of infected cells are activated $CD4^+$ T cells, which die quickly due to viral cytopathic effects or host cytolytic mechanisms (half-life 6 hours to 2 days) (9, 10). On rare occasions, recently activated cells become infected during the transition from an activated state to a resting memory state, which is nonpermissive for viral gene expression. This allows for long-term persistence of stably integrated proviruses in long-lived memory T cells (11). Early studies using a virus culture assay to quantify the frequency of latently infected cells demonstrated that this reservoir is extremely stable (12). A recent study reexamined the half-life of the LR in $CD4^+$ T cells in patients on newer, potentially more effective, cART regimens. The authors estimated a mean reservoir half-life of 3.6 years (13), the same as an original study completed 12 years previously, indicating that newer antiretroviral drugs do not increase the decay rate of the LR. This suggests that the stability of the LR is not due to incomplete suppression of viral replication. This conclusion is also supported by the finding that cART intensification does not cause additional reductions in the trace level of viremia that can be detected in treated patients using sensitive assays (14, 15).

Other Mechanisms of Persistence

Latently infected $CD4^+$ T cells are present throughout the body, wherever memory T cells are found, and constitute the only reservoir yet shown to meet the practical definition given above. However, other anatomical compartments and cellular reservoirs may contribute to HIV persistence. One site of particular interest is the gut-associated lymphoid tissue (GALT). Profound depletion of $CD4^+$ T cells occurs specifically in this compartment during acute infection (16), and

GALT $CD4^+$ levels do not fully recover even after years of suppressive cART (17). In a study of patients on cART, Chun et al. (17) also found evidence of higher levels of HIV proviral DNA in $CD4^+$ T cells isolated from GALT than in $CD4^+$ T cells from peripheral blood.

In addition, HIV can cause central nervous system (CNS) disorders (18), raising concerns that the CNS may harbor HIV in patients on suppressive cART. Immune cells, including microglial cells and perivascular macrophages in the CNS, have the potential to become infected with HIV because these cells express low levels of the protein needed for HIV entry, CD4, and the coreceptor CCR5 (19). In vitro studies have suggested that infected macrophages have a much longer half-life than infected T cells, on the order of weeks instead of hours (10), due to their lower susceptibility to viral cytopathic effects (20). Although the latent infection of macrophages has been modeled in vitro (21), it remains unclear whether replication-competent virus persists in macrophages in the CNS of patients on long-term cART or in other anatomical compartments and, if so, whether the infection is productive or latent. Recent evidence has indicated that macrophages may contain HIV DNA due to their role in the phagocytosis of infected cells, complicating the analysis of macrophage infection (22).

Ongoing Replication

Some investigators have also suggested that ongoing cycles of viral replication continue in patients on cART. The proposed mechanism of this residual replication is by direct cell-to-cell transmission, in which virus may pass directly from an infected cell to an adjacent uninfected cell (23, 24). This mode of viral transmission may not be as susceptible to inhibition by antiretroviral drugs because of the high local multiplicity of infection. Although most studies have suggested that few new infection events occur in patients on cART, some level of cell-to-cell spread may contribute to long-term persistence (25).

DEFINITIONS OF CURE

There are multiple definitions of HIV cure, and each type of cure is being pursued with multiple strategies (**Table 1**). A sterilizing cure is defined as complete eradication of infectious forms of the virus from the body: that is, replication-competent provirus is no longer present. Another possibility is ART-free remission, a scenario in which the HIV reservoir is not eliminated but is reduced enough to greatly decrease the chances of viral rebound. Modeling of infection dynamics has predicted that a 3- to 4-log decrease in the size of the LR is necessary to achieve ART-free remission lasting longer than 1 year (26). A functional cure is thought to be a more attainable goal, as it does not require that the HIV reservoir be reduced or eliminated. Instead, a functional cure allows for discontinuation of cART without rebound because the immune system has been modified in some way so that it is able to control viral replication without therapy (27, 28).

EXAMPLES OF "CURE"

Sterilizing Cure

There is one example of a sterilizing cure. The "Berlin patient," first described in 2008, received an allogeneic hematopoietic stem cell transplant (HSCT) to treat acute myeloid leukemia (29). This case was exceptional because an HLA (human leukocyte antigen)-matched donor was found who was homozygous for the CCR5 Δ32 mutation, which causes a deletion in the CCR5 coreceptor needed for HIV entry. Hence, donor cells were resistant to infection (30). Complete chimerism

Table 1 Definitions and examples of cure

Cure type	Definition	Examples
Sterilizing cure	Complete eradication of replication-competent HIV	Berlin patient: Following bone marrow transplantation from a CCR5 Δ32/Δ32 donor, cART treatment was stopped, and the patient has not experienced viral rebound (30)
ART-free remission	Reduction of HIV reservoir sufficient to greatly increase time to viral rebound when treatment is stopped	Boston patients: These were two individuals who received bone marrow transplantation and achieved complete chimerism with no detectable HIV, but rebounded at 3 and 8 months after cART interruption (31) Mississippi baby: A perinatally infected infant with high viremia at birth began cART very early, but treatment was later discontinued. The infant had viral rebound 26 months after cART interruption (32, 33)
Functional cure	Immune control over HIV infection; does not necessarily require reduction or elimination of HIV reservoirs	Elite controllers: These are individuals who maintain undetectable viral loads without cART due to unique immune control over HIV (36–40) VISCONTI cohort: Initiation of cART during acute infection may have induced immune control in some patients who maintained low-level viremia after cART interruption (41)

Abbreviations: ART, antiretroviral therapy; cART, combination ART; VISCONTI, Virological and Immunological Studies in Controllers after Treatment Interruption.

with the Δ32/Δ32 genotype was achieved 61 days after bone marrow transplantation, meaning that the patient's immune system was almost entirely replaced by donor cells. The patient did not experience viral rebound even though cART was discontinued at the time of transplantation, and HIV DNA could not be detected in plasma or rectal mucosa throughout the follow-up period (29). The Berlin patient is now considered cured, with no detectable HIV present several years later. It is likely that the donor CCR5 Δ32/Δ32 genotype was crucial to the success of this case.

ART-Free Remission

Efforts to reproduce this sterilizing cure in patients receiving allogeneic HSCT have resulted in ART-free remission, but no additional cures. Two patients first described in 2012, referred to as the "Boston patients" (31), received transplants for malignancies from donors with wild-type alleles at the CCR5 locus. Although donor cells were fully susceptible to HIV infection, it was hoped that if the patients maintained cART throughout the transplantation period, donor cells would not become infected, and host cells containing HIV proviruses would be completely eliminated by graft-versus-host disease. The idea was to recapitulate the cure seen in the Berlin patient without the need for an HLA-matched CCR5 Δ32/Δ32 donor. At 2 to 4 years after transplantation, neither patient had detectable HIV DNA in peripheral blood mononuclear cells (PBMC) by polymerase chain reaction (PCR), and replication-competent HIV was not recovered from cocultures. The lack of measurable residual host cells and HIV in these individuals suggested complete elimination of the LR. However, during closely monitored analytical treatment interruptions, the patients experienced viral rebound at 3 and 8 months after discontinuing cART (31).

Unfortunately, it is difficult to apply cure strategies used in the above cases to a typical patient with HIV infection. Despite the promising results seen with some transplantation cases, HSCT is a realistic option only for patients with a concurrent condition requiring this high-risk procedure.

In 2013, ART-free remission was also achieved in a patient known as the "Mississippi baby" (32). In this unusual case, a perinatally infected newborn with a high HIV viral load on the day after delivery was immediately put on cART. At 15 months, therapy was discontinued against medical

advice but, surprisingly, there was no rapid viral rebound (32). Because the child had been started on therapy so early, it was hoped that a reservoir of infected memory $CD4^+$ T cells might not have been established. However, the child presented with high plasma HIV at a routine appointment 26 months after discontinuing cART (33). Subsequent cases of early treatment in perinatally infected infants have had similar results, with viral rebound after treatment interruption (34).

Typically, when a patient discontinues cART, there is a rapid rebound in viremia, beginning within 6 to 15 days, and peaking by 21 days, after interruption of treatment (5). The patients described above achieved ART-free remission for longer periods, a finding with important implications for understanding obstacles to cure. In the Boston patients, little-to-no anti-HIV immunity was present at rebound, consistent with the idea that the donor-derived immune systems that developed in these patients had not experienced HIV before and, therefore, had no HIV-specific immunological memory (31). Therefore, the late rebounds after ART interruption were not due to immune control of viral replication and can be explained only by persistence of virus in a latent form. Modeling of the dynamics of the LR has shown that although a typical pool of latently infected cells can cause rebound within 2 to 3 weeks, a multi-log decrease in this pool can result in a delay from months to years (26). HIV reemerges from a latent state when a $CD4^+$ T cell harboring a latent provirus is reactivated by its cognate antigen or another activating stimulus (35). When the total number of latently infected cells is smaller, at any given time the reemergence of replicating HIV is statistically less likely.

Functional Cure

A small subset of HIV-infected individuals known as elite controllers (ECs) have been viewed as a model for a functional cure, as they are able to maintain low viral loads without therapy and do not progress to AIDS. It was initially thought that ECs were infected with less-virulent strains of HIV, but a study examining virus isolated from transmission pairs has indicated that ECs have this phenotype even when infected with fully functional HIV from a chronic progressor (36). This and several other studies have suggested that ECs are infected with HIV that is fit and virulent, and that these individuals have unique immune control over HIV (37, 38). This control may be related to increased $CD8^+$ T cell responses (39) and, in some patients, to the presence of specific HLA class I alleles (40). Although some HLA alleles are overrepresented in ECs, these do not seem to automatically confer EC status, as patients with progressive disease may also possess these alleles. Because the factors contributing to immune control of HIV in ECs have not been fully characterized, it has proven difficult to develop a therapeutic method to recapitulate this state for a functional cure.

Long-term control of HIV replication without cART was reported in 2013 in the VISCONTI (Virological and Immunological Studies in Controllers after Treatment Interruption) cohort, a small group of patients who began cART early during primary HIV infection and did not experience rebound viremia following treatment interruption (41). Because replication-competent HIV could be isolated from the resting $CD4^+$ T cells of these posttreatment controllers (PTCs), the authors postulated that this effect may have been due to long-term immune control of HIV made possible by early treatment. Notably, the mechanism of control did not appear to be related to HIV-specific $CD8^+$ T cells, although this mechanism has been implicated in ECs' control of HIV infection (39). It is possible that immune control resulted from the preservation of immune response to HIV, which may occur when infected individuals begin treatment early (42).

Although a functional cure may be preferable to daily cART for life, it is important to note that ECs actually have higher levels of immune activation than patients on cART (43). This may result in comorbidities related to chronic inflammation. In fact, it has been reported that ECs are

more likely than HIV-infected individuals on cART to be hospitalized for inflammation-related cardiovascular disorders (44).

STRATEGIES FOR CURE

Early cART

The START (Strategic Timing of Antiretroviral Treatment) trial has documented the definitive health benefits of beginning therapy early in HIV infection. Studies have also demonstrated that starting cART early in infection can result in a smaller reservoir for HIV in $CD4^+$ T cells (45–47). Importantly, beginning cART early does not seem to prevent establishment of the LR. In an SIV (simian immunodeficiency virus) model of HIV infection, rebound following interruption of cART has been demonstrated even when treatment was initiated as early as 3 days after exposure (48). Additionally, the VISCONTI cohort study suggested that early therapy may result in more individuals becoming PTCs, essentially inducing a functional cure (41). A similar phenomenon has also been described in a case study of a patient who began cART early in infection and then underwent low-dose administration of interleukin-2 before treatment interruption (49). This patient also appeared to have posttreatment control of HIV. Elucidation of the mechanism of control by these PTCs may aid further attempts to elicit a functional cure in more individuals. Regardless, the results of these studies suggest that individuals should begin therapy as soon as possible after HIV diagnosis for maximum benefits.

Shock and Kill

The shock-and-kill strategy for HIV eradication aims to directly target latent HIV in resting memory $CD4^+$ T cells and eradicate all remaining replication-competent HIV from the body in patients on suppressive cART (50). Because HIV genes are not actively expressed in latently infected cells, the first step is to shock cells into reinitiating HIV gene expression. When cells begin producing viral antigens, they can potentially be recognized and targeted by an immune response for the kill step.

Multiple classes of small-molecule latency-reversing agents (LRAs) have been identified to potentially initiate the shock step of this strategy (51). Histone deacetylase inhibitors (HDACis), a class of drugs developed to treat certain cancers, are thought to act upon latent HIV by modifying the chromatin state of the long terminal repeat promoter (52), although the mechanism remains controversial (53). HDACis, such as vorinostat and romidepsin, have been studied thoroughly ex vivo and brought into clinical trials with varying levels of success (54–56). In clinical studies employing the shock-and-kill strategy, success in perturbing the LR is assessed by looking for transient increases in cell-associated HIV RNA or viremia to indicate an initiation of HIV gene expression. On a broader scale, success in decreasing the size of the LR may be determined by measuring inducible virus.

To date, no LRA has produced a substantial decrease in the size of the LR. One problem is that induction of HIV gene expression is only half of the strategy. It is also important that the infected cells be eliminated after reversal of latency. Shan et al. (57) have shown that $CD8^+$ T cells from most patients on cART have only limited ability to kill infected cells after reversal of latency. They went on to describe a more successful approach in which $CD8^+$ T cells were prestimulated with Gag peptides to prime the response. These data suggest that some sort of vaccination may be necessary to activate an immune response for the kill step.

Vaccination

There has been a great deal of interest in developing a preventative vaccine for HIV. However, there is also the hope that therapeutic vaccination of infected individuals may enable a functional cure by inducing immune control over HIV replication. Initially, HIV vaccine research focused on envelope-based vaccines to elicit production of broadly neutralizing antibodies (bNAbs). These antibodies act by binding directly to the HIV envelope protein on virus particles and preventing the infection of cells. Inducing these antibodies has proven extremely complicated due to the high degree of sequence variability in the envelope gene and the inaccessibility of stable epitopes (58). Also, bNAbs tend to have unusual structures and arise as a result of a prolonged process of somatic hypermutation reflecting rapid coevolution of the virus and the host antibody response (59). Although various preventative vaccines for HIV have been tested in clinical trials, minimal success has resulted.

Recent studies have reported the isolation from infected patients of highly effective bNAbs against HIV (60). One possible use of bNAbs in HIV infection differs from traditional vaccination approaches that stimulate B cells to produce antibodies against the antigen: this strategy employs passive immunization using the injection of a large quantity of selected bNAb. The bNAb 3BNC117 was tested for inhibitory activity in a phase I clinical trial in HIV-infected patients not on cART (61). The results of this trial indicated that not only is 3BNC117 well tolerated but it also has the potential to reduce a patient's viral load without the use of cART. In some patients this reduction was sustained for weeks to months, due to a much longer half-life of bNAbs compared with antiretroviral drugs. An infusion of bNAbs may be a safe method for delaying viral rebound following cART interruption. Interestingly, in contrast to antiretroviral drugs, which block new infection of susceptible cells, bNAbs can both block new infection and target productively infected cells for destruction by antibody-dependent cell-mediated cytotoxicity (62).

One preventative vaccine, known as RV144, tested in phase III clinical trials in Thailand, has shown some efficacy (31.2%) at preventing infection when compared with infection in a control group (63). In this trial, a bivalent vaccine was designed to induce both antibody and T cell responses. This vaccine was based on HIV clades B and E, which are most prevalent in Thailand. Although correlates with protection, including antibodies to the V1V2 envelope epitopes and the development of antibody-dependent cellular cytotoxicity, were identified, it is not entirely clear whether these elements would be useful in the setting of a therapeutic vaccine (64).

Progress toward a more effective vaccine continues in the SIV–macaque model of HIV infection. A promising approach is vaccination with a rhesus cytomegalovirus (RhCMV) vector that carries SIV genes and induces effector memory T cells specific for SIV proteins. Picker and colleagues (65) reported that 13/24 animals vaccinated with this vector exhibited immune control over SIV infection after intrarectal challenge in contrast to the control group, in which all animals progressed to chronic infection. Long-term follow-up showed that some of the animals receiving the RhCMV–SIV vaccine achieved full eradication of all replication-competent virus (66). It is possible that a strong preexisting $CD8^+$ T cell response to SIV, induced by the vaccine, allowed infected cells to be killed before viral gene expression had been turned off and latency established. This finding is in contrast to the study by Whitney et al. (48), which demonstrated that very early cART does not prevent LR from being established. In that case, it is likely that cells infected prior to the initiation of cART became a part of the LR. Although cART effectively blocks new infection events, it cannot cause infected cells to die. In the vaccine case, a strong preexisting $CD8^+$ T cell response induced by the RhCMV–SIV vaccine may have killed the infected cells before they transitioned to latency (65, 66).

Gene Therapy

Genome editing is an exciting new field that can potentially aid in HIV-cure efforts. Genes can be directly modified in cells using zinc-finger nucleases (ZFN), TALENs (transcription activator-like effector nucleases), or CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 nuclease) technology, all of which target a specific sequence and enable DNA nucleotide editing (67). Multiple approaches to gene therapy in HIV-cure strategies are being studied, including the targeting of HIV entry. One approach is to edit the CCR5 gene in $CD4^+$ T cells to block virus entry (68). The sterilizing cure seen in the Berlin patient, who received a transplant with cells lacking a functional CCR5 gene (29), is the basis for this idea. Edited $CD4^+$ T cells can then be reinfused (68). The major problem with this approach is that after reinfusion, the modified cells represent only a minority of the $CD4^+$ T cell population, and the interruption of cART results in viral rebound. Although a prolonged period of unchecked viral replication would likely selectively deplete unmodified $CD4^+$ T cells, it is not clear that this approach is acceptable, given the associated risks. Editing CCR5 in hematopoietic stem cells is also being considered, and this approach may have an exciting potential use for patients requiring bone marrow transplantation because the CCR5 $\Delta 32/\Delta 32$ phenotype that renders cells resistant to HIV infection is extremely rare and it is difficult to find matched donors homozygous for this mutation. If normal donor cells can be edited before they are given, it may be possible to induce this phenotype during allogeneic HSCT.

Another approach is to use gene-editing technology to directly target the HIV provirus. A recent study using J-Lat, a cell line used to model latent HIV, has shown that CRISPR/Cas9 can be used to cleave and mutate integrated HIV DNA (69). CRISPR/Cas9 has similarly been shown to potentially cleave HIV provirus entirely out of the genome (70). This approach has the potential to inactivate latent provirus in patients on suppressive cART, effectively reducing the pool of replication-competent HIV that remains for the long term. However, this would require high-efficiency delivery of the editing enzymes into every infected cell in vivo, something that would be difficult to achieve. At this point, genome-editing strategies are still very new, and concerns remain about the safety and off-target effects. One clinical trial studying the adoptive transfer of $CD4^+$ T cells with ZFN-edited CCR5 genes has demonstrated that this intervention was well tolerated (68).

An alternative approach to genome editing has focused on enhancing the immune response to HIV-infected cells to potentially control infection without the need for cART. This includes attempts to introduce HIV-specific T cell receptor genes to $CD8^+$ T cells to allow for more widespread recognition of infected cells, better control of viral replication, and to possibly induce a functional cure (71).

CURE RESEARCH: HOW TO MEASURE SUCCESS

Assays to Measure the Latent Reservoir

A sterilizing cure or prolonged ART-free remission will require a drastic decrease in the pool of latently infected $CD4^+$ T cells. Latently infected cells are rare, with an average frequency of about 1 cell with replication-competent virus per 10^6 resting $CD4^+$ T cells (8, 12). For this reason, measuring the LR is difficult. There is no clinical assay for the LR. It is important to have an accurate method for measuring a change in the size of the LR in patients undergoing cure interventions.

The gold standard for measuring the LR is the viral outgrowth assay (VOA), in which limiting dilutions of resting $CD4^+$ T cells are stimulated with an activating mitogen in culture, and the

amount of infectious virus produced is measured by HIV p24 antigen ELISA (enzyme-linked immunosorbent assay) (8, 72). This assay has the advantage of detecting only replication-competent virus, but has recently been shown to underestimate the size of the LR because some replication-competent proviruses are not induced after a single round of T cell activation in this assay (73). VOA also requires a large number of cells and 2 to 3 weeks of cell culture, rendering it more expensive and time-consuming than PCR-based methods.

Multiple PCR-based methods have been developed to more easily measure the size of the LR. Detection of HIV DNA in unfractionated PBMC or purified resting $CD4^+$ T cells can be used to quantify the total number of cells carrying an HIV provirus (74, 75). A major caveat of this method is that it detects defective proviruses, which do not pose a barrier to cure. This discrepancy can lead to an overestimation of the size of the LR by as much as 300-fold relative to estimates based on the VOA (76).

PCR detection of induced HIV RNA can also be used to measure the LR. This can be done with a T cell activation protocol similar to that used in the VOA, with the ultimate readout being quantitative reverse transcriptase-PCR detection of HIV RNA instead of ELISA (77). This method is less time-consuming than VOA and more accurate than detecting proviral DNA, but it may pick up some defective proviruses that can be transcribed but cannot produce infectious virus. Quantifying HIV RNA transcripts can also be useful in identifying effective LRAs for the shock-and-kill strategy, as this method can measure changes in HIV expression in patient cells treated ex vivo (78, 79).

Analytic Treatment Interruptions

When moving potentially curative treatment options to the clinic, one feasible method of analyzing long-term effects is analytic treatment interruption (ATI). ATI refers to interrupting cART to measure the time until plasma HIV is detectable. If the time is longer than what is typically seen without additional cure interventions (21 days to peak viremia), it can be inferred that the intervention may have had an effect on the size of the LR. An ATI directly tests whether an ART-free remission is possible, either from reservoir reduction or immune control. This is in contrast to other assays measuring the size of the LR: They are unable to measure alterations in the immune response that might allow control of HIV replication.

Although an ATI is the only way to truly determine the efficacy of a cure strategy, the potential clinical consequences of interrupting cART bring the ethics of such a strategy into question. There can be risks associated with interrupting cART, including the potential for immune deficiency as well as cardiovascular, renal, or hepatic disease (80). These problems are much less likely to occur if a patient is closely monitored during an ATI and put back on therapy as soon as viremia returns. Another concern regarding ATIs is that drug-resistance mutations can arise when therapy is stopped or restarted improperly. In a large-scale study involving 688 patients who required interruption of cART regimens using non-nucleoside reverse-transcriptase inhibitors, only 87.4% of patients were able to restart treatment using the same regimen, while the rest had developed resistance mutations (81). Despite these concerns, a recent study of carefully monitored ATIs found no safety issues (82). ATIs should always be initiated with caution and close monitoring of patients to minimize risks. This is particularly important for patients who have received HSCT because of the risk of symptomatic, acute retroviral syndrome (31).

CONCLUSIONS

Recent reports have renewed optimism about the potential for curing HIV infection. Unfortunately, these cases do not represent the broad cure strategy that is necessary to impact the global

HIV epidemic because they cannot be applied to the vast majority of individuals living with HIV. Careful evaluation of these case studies gives hope that a cure is possible, and they continue to inform new research strategies that bring us closer to a cure.

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